VIRAL HEPATITIS

Apolipoprotein $\epsilon 3$ allele is associated with persistent hepatitis C virus infection

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Background: Host genetic factors may significantly influence the ability to clear hepatitis C virus (HCV) following infection. HCV is associated with very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the host's circulation. Apolipoprotein E (APOE) is found in VLDL and binds to potential receptors involved in HCV entry into cells, the LDL receptor, and the scavenger receptor protein SR-B1. The APOE gene is polymorphic with three alleles coding for three isoforms: Apo- $\epsilon 2$, Apo- $\epsilon 3$, and Apo- $\epsilon 4$. The aim of this study was to assess if these functional polymorphisms determine disease outcome in HCV infected individuals.

Methods: The APOE genotype was determined in 420 Northern European patients with evidence of exposure to HCV. Genotype and allele distribution were compared with those of 288 healthy controls and progression of liver disease and viral clearance were analysed according to APOE allele status.

Results: The APOE*E2 and APOE*E4 alleles were both associated with a reduced likelihood of chronic infection (odds ratio (OR) 0.39 (95% confidence interval (CI) 0.211–0.728), $p=0.003$; and OR 0.6 (95% CI 0.38–0.96), $p=0.032$) and there was a notable absence of the E2E2 genotype in the HCV antibody positive group compared with the control population ($p=0.0067$). Overall the genotypes carrying the E2 allele (E2,E3 and E2,E4) were associated with the equivalent of a 3–5-fold reduction in the risk of chronic HCV infection (genotype relative risk 0.36 and 0.20, respectively).

Conclusion: This study indicates that functional APOE gene polymorphisms may be a determinant of outcome in HCV infection. We hypothesise that the E2 allele may protect against viral persistence via defective binding of HCV lipoviral particles to the cellular receptors involved in entry of these infectious particles.

Hepatitis C virus (HCV) infection is a major global health problem, infecting more than 170 million people worldwide.¹ Some newly infected patients recover spontaneously but the majority progress to chronic infection so that HCV infection is now the leading cause of cirrhosis, hepatocellular cancer, and liver transplantation in the Western world.²

Persisting strong CD8+ T cell responses are observed in the majority with resolved HCV infection³ but the role of host factors in viral clearance and disease progression remains unclear. There is some evidence that host genetic factors are implicated in the outcome following HCV infection.⁴ Specific HLA haplotypes have been associated with a significantly increased likelihood of viral clearance, including the HLA-DRB1*1101-DQB1*0301, DRB1*1104-DQB1*0301, and DRB1*0401-DQB1*0301 haplotypes.^{4–7} In addition, recent data suggest that inherited variation in the killer inhibitory receptor genes may play a significant role in determining the host response to HCV infection.⁸

Other host factors associated with disease progression in chronic HCV include male sex, age at infection, excessive alcohol use, total cholesterol level, and insulin resistance.⁹ In addition, there is now considerable accumulating evidence to suggest a relationship between hepatitis C virus and lipid metabolism.¹⁰ During the early stages of acute HCV infection in chimpanzees, host genes involved in lipid metabolism are differentially regulated.¹¹ In patients with chronic HCV, serum cholesterol is significantly lower than in appropriately matched controls,¹² particularly in HCV genotype 3 infection,^{13 14} and this metabolic effect is fully reversible after successful HCV eradication.¹⁵ The association of HCV infection

with hypocholesterolaemia has been confirmed in human immunodeficiency virus (HIV) infected patients¹⁶ and in patients with porphyria cutanea tarda.¹⁷ In many studies the hypocholesterolaemia has been associated with a decrease in apolipoprotein B (ApoB) in comparison with healthy controls^{12 17 18} and ApoB levels negatively correlate with hepatic steatosis and viral load.¹⁸ Furthermore, HCV viral load can be reduced by heparin induced extracorporeal lipoprotein fibrinogen precipitation apheresis, which is an established and approved therapy for hypercholesterolaemia.¹⁹

HCV RNA containing particles are consistently found in the low density fractions of serum and this fraction has been shown to be highly infectious.²⁰ Further characterisation of these low density HCV RNA containing particles (lipoviral particles (LVP)) has shown that they efficiently bind and enter hepatocyte cell lines, upregulation of the low density lipoprotein receptor (LDLr) increases their internalisation, and binding of HCV-LVP can be blocked by anti-ApoB and anti-apolipoprotein E (ApoE).^{21 22} This is consistent with other studies showing that endocytosis of HCV can be mediated by LDLr.^{23 24} LDLr, a candidate receptor for HCV, normally transports two different classes of cholesterol containing lipoprotein particles into cells: LDL, which contains a single copy of ApoB-100, and very low density lipoprotein (VLDL), which contains multiple copies of ApoE.

Abbreviations: HCV, hepatitis C virus; VLDL, very low density lipoprotein; LDL, low density lipoprotein; LDLr, low density lipoprotein receptor; ApoE, apolipoprotein E; APOB, apolipoprotein B; LVP, lipoviral particle; PCR, polymerase chain reaction; IVDU, intravenous drug user; HIV, human immunodeficiency virus

Recent studies with retrovirus/HCV pseudovirus particles²⁵ suggest that attachment to hepatocytes is via the scavenger receptor protein, SR-B1, with entry into the cell via a co-receptor CD-81 dependent pathway.^{26–27} SR-B1, which is expressed primarily in the liver, is a key component in the reverse cholesterol transport pathway, and recognises a broad variety of lipoprotein ligands, including high density lipoprotein, LDL, VLDL, and oxidised LDL.^{28–30}

ApoE, a ligand for both LDLr and SR-B1, is a polymorphic protein arising from three alleles at a single gene locus. The three major isoforms, Apo-ε2, Apo-ε3, and Apo-ε4, differ from one another by single amino acid substitutions, a change which has profound functional consequences at both the cellular and molecular levels. Apo-ε3 seems to be the wild-type isoform with normal function, while Apo-ε2 binds poorly to LDLr, and Apo-ε4 induces downregulation of LDLr.³¹

A previous study of *APOE* gene polymorphisms in HCV infection suggested decreased severity of liver disease in patients with the *E4* allele.³² As ApoE is a ligand for the HCV receptor candidates LDLr and SR-B1 and in view of the considerable evidence suggesting that cholesterol and fatty acid pathways may play a role in HCV replication and infection, the aim of this study was to determine whether the common functional polymorphisms of the *APOE* gene can influence the clinical outcome of HCV infection.

PATIENTS AND METHODS

Patients

Whole blood (5 ml) was obtained from 420 Caucasian HCV antibody positive patients recruited from two centres, the joint hepatitis clinic of Freeman Hospital, Newcastle upon Tyne, UK (n = 241) and St James's Hospital, Dublin, Ireland (n = 179).³³ Whole blood (5 ml) was also collected from 288 healthy adult volunteers (controls) to assess the ApoE genotype distribution in the healthy population.³⁴

HCV viral RNA was tested in all HCV antibody positive patients using the Amplicor reverse transcriptase polymerase chain reaction (PCR) assay (Roche, Lewes, East Sussex, UK). A total of 312 patients were HCV RNA positive on at least two occasions, indicating persistent or chronic infection, whereas 108 patients were HCV RNA negative on at least two occasions, indicating spontaneously resolved infection.

Liver biopsies were available for 209 patients with evidence of chronic infection. Each biopsy was scored by a single observer, Professor AD Burt, using the Ishak scoring system.³⁵ Biopsies were graded for necroinflammation on a scale of 0–16 and fibrosis on a scale of 0–6 (fibrosis stage 5 or 6 indicated severe disease, 2–4 moderate disease, and 0 or 1 mild disease). An estimate of the rate of fibrosis was made on 178 of these patients. Date of infection was either estimated in intravenous drug users (IVDU) as the date when IVDU started, or was calculated as the date of exposure to infected blood or the date of a one off/short period of IVDU. The Ishak stage of fibrosis was divided by the time in years from the date of infection to first biopsy to give a calculation of the rate of fibrosis. Rapid progression was identified as patients progressing to cirrhosis within 20 years (a rate of fibrosis >0.25/year). In this subgroup of patients, data on alcohol use, age at biopsy, sex, and mode of infection were also collected; all data and results were entered into a Microsoft Access database.

Apolipoprotein E genotyping

Genomic DNA was isolated from 2 ml of whole blood using QIAamp midi kits (Qiagen Ltd, Crawley, UK). A 227 bp product of the *APOE* gene was amplified using a pair of sequence specific primers in a standard PCR reaction.³⁶ Briefly, approximately 100 ng of DNA were amplified in a

50 µl reaction mix containing 2.5 µl of 100% DMSO, 1.5 mM MgCl₂, 10 mM Tris-HCl, 50 mM KCl, 0.5 µM of each primer, 200 µM of each nucleotide, and 2.5 U of *Taq* polymerase (Abgene, Epsom, Surrey, UK). Amplification conditions were as follows: two minutes at 95°C and then 40 cycles of two minutes at 95°C, two minutes at 65°C, and two minutes at 72°C, with a final extension for five minutes at 72°C. All PCR batches included a water (DNA free) negative control and samples of known genotype. All genotypes were assigned by two independent investigators and any ambiguous genotypes were repeated. The 227 bp product was subaliquoted into duplicate reaction tubes and subjected to digestion with two different restriction endonucleases *AflIII* and *HaeII*; digestions were performed with 25 U of each enzyme at room temperature for a minimum of three hours. The resulting restriction fragments were separated on 3% agarose gels and the bands were visualised using ethidium bromide staining on a UV transilluminator and documented on an AlphaImager 2200 Documentation and Analysis System (AlphaInnotech Corp., USA). *AflIII* digests the PCR amplicons of the *APOE**2 and *E3* alleles only creating bands of 117 bp and 50 bp (amplicons of *E4* do not digest, leaving the 227 bp amplicon intact). *HaeII* digests the *E3* and *E4* amplicons only resulting in bands of 195 bp and 32 bp (amplicons of the *E2* allele are not digested, leaving an intact 227 bp amplicon). Thus by comparing the restriction patterns for the two digests on every sample, we can assign composite genotypes for *E2,E2*; *E2,E3*; *E3,E3*; *E2,E4*; *E3,E4*; and *E4,E4*.

Statistical analysis

Genotype and allele distribution were compared using Stata. As the *E3* allele is associated with normal *APOE* activity, the risk for the common (wild-type) genotype *E3,E3* was fixed (at 1) and the risk for all other genotypes are reported relative to this. In the whole HCV antibody positive cohort (n = 420) the following comparisons of allele and genotype distribution were made:

- control group (n = 288) versus HCV exposed (HCV antibody positive) population;
- chronic HCV infection (n = 312) versus spontaneously resolved (cleared) infection (n = 108).

In the subgroup with chronic HCV infection whose liver biopsies were scored by a single observer, the following comparisons of allele and genotype distribution were made:

- severe liver disease versus mild/moderate chronic hepatitis (n = 209);
- slow fibrosis progression versus rapid fibrosis progression (n = 178).

Risk is reported as genotype relative risk (GRR) for comparison of genotype distribution, and odds ratio (OR) for comparison of allele distribution with 95% confidence intervals (CI). Multivariate binary logistic regression was used to assess independent variables, including sex, alcohol usage, and age of infection in relation to *APOE* status in HCV antibody positive patients. All additional computations were performed using SPSS-10 for windows.

RESULTS

Comparing all patients versus healthy controls, there was a significantly lower frequency of the *E2,E2* genotype in the diseased population ($\chi^2 = 7.34$, $p = 0.0067$) (table 1).

Comparison of the *APOE* genotypes in the two clinical subgroups (table 2) of patients with spontaneously resolved infection (HCV RNA negative cleared infection, n = 108) versus chronic infection (HCV RNA positive chronic infection, n = 312) demonstrated that the genotypes *E2,E3* and *E2,E4*

Table 1 Apolipoprotein B (APOE) genotype and allele distribution (number (%)) for hepatitis C virus (HCV) infected patients and healthy controls

APOE genotype	Controls (n = 288)	HCV cohort (n = 420)	p Value
E2,E2	5 (2%)*	0*	0.0067
E2,E3	32 (11%)	39 (9%)	NS
E2,E4	11 (4%)	9 (2%)	NS
E3,E3	167 (58%)	263 (63%)	NS
E3,E4	66 (23%)	105 (25%)	NS
E4,E4	7 (2%)	4 (1%)	NS

were mostly strongly associated with clearance of HCV ($p = 0.005$, GRR $E2,E3$ 0.36 (95% CI 0.18–0.73); and $p = 0.02$, GRR $E2,E4$ 0.2 (95% CI 0.52–0.78), respectively). In addition, while there was a significant effect of the $E3,E4$ genotype ($p = 0.022$, GRR 0.55 (95% CI 0.33–0.92)) the $E4,E4$ genotype was not significant ($p = 0.811$) due to the low numbers in the comparator subgroups. Comparing allele distribution, the lowest risk of chronic infection was associated with the $E2$ allele (OR 0.39 (95% C.I. 0.21–0.73)) compared with the $E4$ allele (OR 0.59 (95% CI 0.38–0.96)).

$APOE^*2$ was the only independent predictor of viral clearance in a multivariate logistic regression model. No other significant associations were found comparing patients and controls, or comparing those with chronic versus those with resolved HCV infection. In addition, there was no significant association between $APOE$ genotype or $APOE$ alleles and the clinical phenotypes defined by severe inflammation (Ishak grade >8 , $n = 37$), severe fibrosis (Ishak stages 5 and 6, $n = 45$), or fast fibrosis (rate of fibrosis ≥ 0.25 /year, $n = 60$).

DISCUSSION

ApoE plays an important role in the transport of cholesterol and other lipids among cells of various tissues. Three major isoforms, encoded by three common alleles at the $APOE$ locus, have different binding affinities for the Apo E receptors. The $E2$ allele is associated with defective binding to LDLr and the $E4$ allele with downregulation of LDLr compared with $E3$.³¹ In this study, $APOE^*2$ and $APOE^*4$ alleles were both associated with an increased likelihood of viral clearance and, interestingly, there were no HCV antibody positive patients with the $APOE$ $E2,E2$ genotype, although this is relatively rare in healthy controls (0.5–2%).³⁷ A previous investigation of the influence of $APOE$ polymorphism and outcome of HCV infection³² suggested that the $APOE^*4$ allele protected against severe liver disease. Our results did not confirm this finding but suggested that this allele may also be associated with a reduced risk of viral persistence. However, it is probable that neither this nor the

earlier study was adequately powered to look at subtle effects of the Apo- $\epsilon 4$ isoform on disease progression. This latter comment is especially true when other factors associated with disease progression, which may vary between cohorts, are considered.⁹ Another small study has suggested that heterozygosity for the $APOE^*4$ allele might be associated with better histological outcome in recurrent HCV infection in the liver transplantation setting³⁸ but this situation is complicated by possible differences in donor/recipient $APOE$ alleles. It is also of interest that chronic HCV is associated with cognitive dysfunction and CNS infection³⁹ and a recent study has demonstrated an association between $APOE^*4$ and neuropsychiatric symptoms during interferon alpha treatment for chronic HCV.⁴⁰

The association found between the $E2$ allele and viral clearance is intriguing as this allele binds poorly to LDLr.³¹ It is plausible that such defective binding could result in poor uptake of HCV lipoviral particles into hepatocyte with a resultant decrease in replication of the virus, thus altering the balance between virus replication and the immune response and favouring clearance of the virus before chronic infection can be established. This is supported by the lack of $E2,E2$ genotype among patients who are HCV antibody positive, suggesting that the $E2,E2$ genotype (0.5–2% of healthy controls³⁷) may confer relative resistance to establishing HCV infection in exposed individuals. This could be somewhat analogous to the role played by the chemokine receptor-5 delta 32 mutation, which leads to defective uptake of the HIV virion and therefore resistance to HIV/acquired immunodeficiency syndrome.⁴¹ In the latter case, homozygotes for the delta 32 mutation exhibit a strong, although incomplete, resistance to HIV infection whereas heterozygotes display delayed progression to acquired immunodeficiency syndrome. Conversely, the results reported here may indicate an association between the $APOE^*3$ allele and persistence of the hepatitis C virus. This would fit a model whereby $APOE^*3$ binds to receptors with high affinity and is associated with normal serum cholesterol and triglyceride levels.

In conclusion, this study suggests that common genetic variations at the $APOE$ locus influence the outcome of HCV infection, with the $APOE^*E2$ and $APOE^*E4$ alleles favouring viral clearance. We hypothesise that ApoE2, which binds poorly with its receptors, may be associated with resistance to HCV infection via defective uptake of HCV lipoviral particles by the candidate receptors LDLr and SR-B1. Further investigations are needed, especially in individuals who are likely to have been repeatedly exposed to HCV but remain anti-HCV antibody negative.⁴² Although the inability to replicate the results of case control studies has led to scepticism about their value,⁴³ our findings are both biologically plausible and statistically significant. If our findings are confirmed in a validation study, the function of apolipoprotein E in assembly, processing, and removal of plasma lipoproteins

Table 2 Apolipoprotein E (APOE) genotype distribution in patients with spontaneously resolved infection (HCV RNA negative cleared infection) versus chronic infection (HCV RNA positive chronic infection)

APOE genotype	Chronic HCV infection (n = 312)	Cleared HCV infection (n = 108)	GRR (95% CI)	p Value
E2,E2	0	0	–	–
E2,E3	23 (7.5%)	16 (15%)	0.36 (0.18–0.73)	0.005
E2,E4	4 (1%)	5 (4%)	0.20 (0.52–0.77)	0.020
E3,E3	210 (67.5%)	53 (49%)	1	–
E3,E4	72 (23%)	33 (31%)	0.55 (0.33–0.92)	0.022
E4,E4	3 (1%)	1 (1%)	0.76 (0.77–7.42)	NS

Values are number (%).

HCV, hepatitis C virus; GRR, genotype relative risk; 95% CI, 95% confidence interval.

and its interaction with HCV could be considered in the development of novel therapeutic strategies.

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REFERENCES

- 1 Poyndar T, Yuen MF, Ratzliff V, et al. Viral hepatitis C. *Lancet* 2003;362:2095-100.
- 2 Alter HJ, Seeff LB. Recovery, persistence and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis* 2000;20:17-35.
- 3 Lauer GM, Barnes E, Lucas M, et al. High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection. *Gastroenterology* 2004;127:924-36.
- 4 Yee LJ. Host genetic determinants in hepatitis C virus infection. *Genes Immun* 2004;5:237-45.
- 5 Donaldson PT. Genetics of liver disease: immunogenetics and disease pathogenesis. *Gut* 2004;53:599-608.
- 6 Cramp ME, Carucci P, Underhill J, et al. Association between HLA class II genotype and spontaneous clearance of hepatitis C viraemia. *J Hepatol* 1998;29:207-13.
- 7 Thursz M, Yallop R, Goldin R, et al. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. *Lancet* 1999;354:2119-24.
- 8 Khakoo SI, Thio CL, Martin MP, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 2004;305:872-4.
- 9 Sud A, Hui JM, Farrell GC, et al. Improved prediction of fibrosis in chronic hepatitis C using measures of insulin resistance in a probability index. *Hepatology* 2004;39:1239-47.
- 10 Andre P, Perlemuter G, Budkowska A, et al. Hepatitis C virus particles and lipoprotein metabolism. *Semin Liver Dis* 2005;25:93-104.
- 11 Su AI, Pezacki JP, Wodicka L, et al. Genomic analysis of the host response to hepatitis C virus infection. *Proc Natl Acad Sci U S A* 2002;99:15669-74.
- 12 Serfaty L, Andreani T, Giral P, et al. Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. *J Hepatol* 2001;34:428-34.
- 13 Sharma P, Balan V, Hernandez J, et al. Hepatic steatosis in hepatitis C virus genotype 3 infection: does it correlate with body mass index, fibrosis, and HCV risk factors? *Dig Dis Sci* 2004;49:25-9.
- 14 Hofer H, Banki HC, Wrba F, et al. Hepatocellular fat accumulation and low serum cholesterol in patients infected with HCV-3a. *Am J Gastroenterol* 2002;97:2880-5.
- 15 Poyndar T, Ratzliff V, McHutchison J, et al. Effect of treatment with peginterferon or interferon alpha-2b and ribavirin on steatosis in patients infected with hepatitis C. *Hepatology* 2003;38:75-85.
- 16 Polgreen PM, Fultz SL, Justice AC, et al. Association of hypocholesterolaemia with hepatitis C virus infection in HIV-infected people. *HIV Med* 2004;5:144-50.
- 17 Fernandez MC, de-la-Calle M, Gomez IMT, et al. Serum lipoproteins in patients with porphyria cutanea tarda. *Med Clin (Barc)* 1999;112:656-7.
- 18 Petit JM, Benichou M, Duvillard L, et al. Hepatitis C virus-associated hypobetalipoproteinemia is correlated with plasma viral load, steatosis, and liver fibrosis. *Am J Gastroenterol* 2003;98:1150-4.
- 19 Schettler V, Monazahian M, Wieland E, et al. Reduction of hepatitis C virus load by H.E.L.P.-LDL apheresis. *Eur J Clin Invest* 2001;31:154-5.
- 20 Penin F, Dubuisson J, Rey FA, et al. Structural biology of hepatitis C virus. *Hepatology* 2004;39:5-19.
- 21 Andre P, Komurian PF, Deforges S, et al. Characterization of low- and very-low-density hepatitis C virus RNA containing particles. *J Virol* 2002;76:6919-28.
- 22 Agnello V, Abel G, Elfahal, et al. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc Natl Acad Sci U S A* 1999;96:12766-71.
- 23 Monazahian M, Bohme I, Bonk S, et al. Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. *J Med Virol* 1999;57:223-9.
- 24 Wunschmann S, Medh JD, Klinzmann D, et al. Characterization of hepatitis C virus (HCV) and HCV E2 interactions with CD81 and the low-density lipoprotein receptor. *J Virol* 2000;74:10055-62.
- 25 Bartenschlager R. The hepatitis C virus replicon system: From basic research to clinical application. *J Hepatol* 2005;43:210-16.
- 26 Bartosch B, Vitelli A, Granier C, et al. Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. *J Biol Chem* 2003;278:41624-30.
- 27 Lavillette D, Tarr AW, Voisset C, et al. Characterization of host-range and cell entry properties of the major genotypes and subtypes of hepatitis C virus. *Hepatology* 2005;41:265-74.
- 28 Calvo D, Gomez-Coronado D, Lasuncion MA, et al. CLA-1 is an 85-kD plasma membrane glycoprotein that acts as a high affinity receptor for both native (HDL, LDL, and VLDL) and modified (OxLDL and AcLDL) lipoproteins. *Arterioscler Thromb Vasc Biol* 1997;17:2341-9.
- 29 Rhoads D, Brodeur M, Lapointe J, et al. The role of human and mouse hepatic scavenger receptor class B type 1 (SR-B1) in the selective uptake of low-density lipoprotein-cholesteryl esters. *Biochemistry* 2003;42:7527-38.
- 30 Favre D, Muellhaupt B. Potential cellular receptors involved in hepatitis C virus entry into cells. *Lipids Health Dis* 2005;4:9-14.
- 31 Mahley RW, Rall SCJ. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 2000;1:507-37.
- 32 Wozniak MA, Itzhaki R, Faragher EB, et al. Apolipoprotein E-epsilon 4 protects against severe liver disease caused by hepatitis C virus. *Hepatology* 2003;36:456-63.
- 33 Kenny-Walsh E, Group IHR. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Engl J Med* 1999;340:1228-33.
- 34 McIlroy SP, Vahidassr MD, Savage DA, et al. Risk of Alzheimer's disease is associated with a very low-density lipoprotein receptor genotype in Northern Ireland. *Am J Med Genet* 1999;88:140-4.
- 35 Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696-9.
- 36 Wenham PR, Price WH, Blandell G. Apolipoprotein E genotyping by one-stage PCR. *Lancet* 1991;337:1158-9.
- 37 Hallman DM, Boerwinkle E, Saha N, et al. The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. *Am J Hum Genet* 1991;49:338-49.
- 38 Tonuito P, Fabris C, Fumo E, et al. Carriage of the apolipoprotein E-epsilon4 allele and histological outcome of recurrent hepatitis C after antiviral treatment. *Am J Clin Pathol* 2004;122:428-33.
- 39 Forton DM, Taylor-Robinson SD, Thomas HC. Cerebral dysfunction in chronic hepatitis C infection. *J Viral Hepat* 2003;10:81-6.
- 40 Gochee PA, Powell EE, Purdie DM, et al. Association between apolipoprotein E epsilon4 and neuropsychiatric symptoms during interferon alpha treatment for chronic hepatitis C. *Psychosomatics* 2004;45:49-57.
- 41 Blanpain C, Libert F, Vassart G, et al. CCR5 and HIV infection. *Receptors Channels* 2002;8:19-31.
- 42 Freeman AJ, French RA, Post JJ, et al. Prevalence of production of virus-specific interferon-gamma among seronegative hepatitis C-resistant subjects reporting injection drug use. *J Infect Dis* 2004;190:1093-7.
- 43 Colhoun HM, McKeigue, Smith GD. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003;361:865-72.